Non-invasive prenatal testing for an affected fetus with beta thalassemia

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Background and Objective: Non-invasive prenatal testing (NIPT) of maternally inherited alleles of β-thalassemia continues to be challenging to find suitable techniques available for use as routine tests. NIPT for β-thalassemia disease in the present study was developed by utilizing a specific droplet digital polymerase chain reaction (ddPCR) assay to analyze the cell-free fetal DNA (cffDNA) derived from maternal plasma.

Study designs: ddPCR assay sets were constructed for the 4-common beta-thalassemia mutations (CD 41/42-TCTT, CD17A> T, IVS1-1G>T and CD26G>A). The assay sets were validated according to the protocol described by Droplet Digital PCR Bio-Rad QX 100 System.

Material and Method: Fifty-two couples at risk of having a baby with compound heterozygous (CHBT) and three homozygous β-thalassemia (HBT) were enrolled at Phayao Hospital between 2018-2021. The gestational age at the time of study was between 10 and 16 weeks. Maternal plasma cell-free DNA was isolated. All cell-free DNA samples were firstly screened for paternally inherited beta-thalassemia (PIB) mutations using a paternal-specific ddPCR assay. Twenty-six samples were PIB-negative considering non-disease, and were not further analyzed. In the remaining PIB positive samples, DNA fragments 50-300 bp in size were isolated and purified by an automated DNA sizing selection approach and were further analyzed for maternally inherited beta-thalassemia (MIB) mutations. The allelic ratio between the mutant and the wild type was used to determine the present of MIB in cffDNA.

Results: Fifty-five couples were enrolled. Twenty-nine cases were positive for PIBs. Among these 29 samples, there were 15 cases with allelic ratio ≥ 1 (MIB positive). The formulated diagnoses of these overrepresented mutant alleles revealed 13 CHBT, and two fetuses were HBT. The 26 PIB-negative and 14 MIB-negative fetuses were non- affected fetuses. The results were in concordance with that of prenatal diagnosis by amniocentesis.

Conclusion: We conclude that NIPT using the 4-constructed ddPCR assay sets can be used for the identification of MIB and PIB of beta-thalassemia fetus in at-risk pregnancies. The method is simple, accurate, and beneficial for beta-thalassemia prevention programs.
Foot note: Part of the result has been accepted for publication in Indian Journal of Medical Research.

Keywords: 1. non-invasive prenatal testing 2. droplet digital polymerase chain reaction assay 3. maternally inherited beta-thalassemia and paternally inherited beta-thalassemia test